CHROMOSOME VARIATION AND REPRODUCTIVE SYSTEM IN A POPULATION OF SESSILE-FLOWERED *TRILLIUM RECURVATUM*

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(Received January 20, 1988)

Many sessile-flowered *Trillium* species grow in the southeastern United States of America, but there are no sessile-flowered species in eastern Asia. Although the present author has done chromosome analyses of *T. erectum*, *T. grandiflorum*, *T. ovatum*, and *T. kamtschaticum* in the past three decades, all the above species are pedicellate-flowered *Trilliums* (in *T. erectum*-FUKUDA, 1987; in *T. grandiflorum*-FUKUDA and GRANT, 1980; in *T. ovatum*-FUKUDA and CHANNELL, 1975; in *T. kamtschaticum*-FUKUDA and KOZUKA, 1958, FUKUDA, 1970). *Trillium recurvatum* Beck. is a typical sessile-flowered species (Figs. 1 and 2, FREEMAN, 1974). This species distributed in moist forests from Michigan and Wisconsin in the north to Tennessee and Arkansas in the south (Fig. 3). The plants bloom in April and May in a spring season and make fruits in July in a summer season.

This article presented chromosome-banding information of *T. recurvatum* from the Meeman-shelby Forest, Shelby Co., Tennessee, where field observation has done in the flowereing period, and discusses the characteristics of the sessile-flowered *Trillium*.

Materials and Methods

The materials of the populations samples were collected from the Meeman-shelby Forest near Memphis, Tennessee, in the spring season of 1987 (Fig. 3). When the rhizomes collected were transplanted into pots, they developed roots in June. After the experiment has finished, all plants were returned to the same habitat at the forest. Now, after sufficient root developed, the plants were placed in a refrigerator at the Genetics Laboratory, Rhodes College, Memphis, Tennessee, for pre-treatment of the Feulgen reaction. The temperature of the refrigirator was kept at 0°C–1°C for 96 hours. Fixation was done in LaCour 2BE for 15 minutes. After hydrolysis at 60°C for minutes in a water bath, the Feulgen solution was used to stain the chromosome-bandings.

Fig. 1. *T. recurvatum* plants at the Meeman-Shelby Forest in Tennessee.

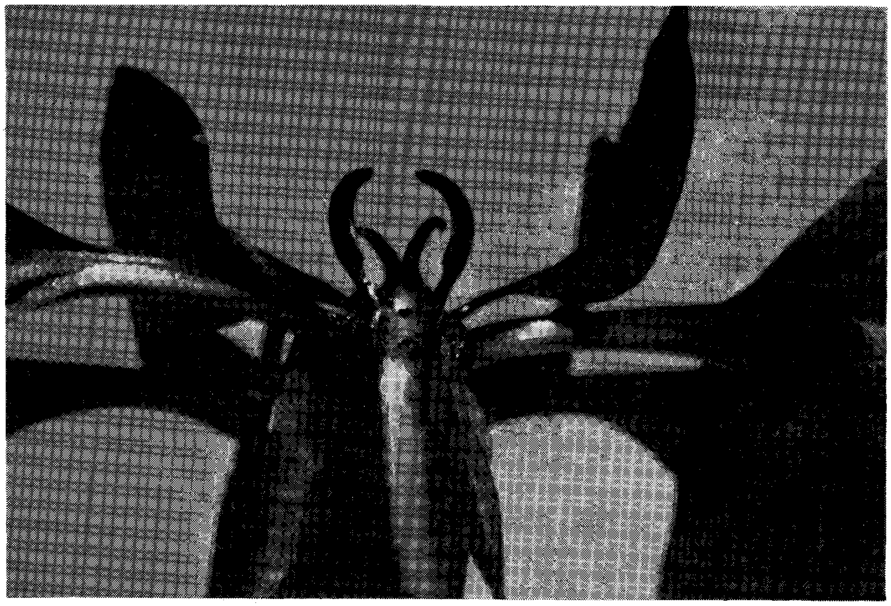

Fig. 2. A sectioned flower of *T. recurvatum* showing a pistil and stamens when the flower has opened.

Chromosome examinations were done of the meristem cells of the root tips from rhizomes. The preparations were made by a squash method with mounting done in 45% acetic acid. The chromosome-banding pattern for an individual plant was determined by observations of over 50 cells for from six to ten preparations from each plant. The sketches of the chromosome figures for each sample were drawn by using a camera lucida (Fig. 4).

The chromosome composition in a population was determined by mean of the kinds of chromosomes and the type numbers of the chromosome-bandings (Fig. 5 and Table 1).

Chromosome Analyses *T. recurvatum*

(1) Chromosome Types-Kinds of Chromosome-banding Patterns

The induced chromosome-banding patterns after cold treatment are illustrated in Fig. 4. Each plant of *T. recurvatum* contains of a pair A, B, C, D and E chromosomes. All plants in the population also have one or two supernumerary chromosomes (Table 1).

Table 1. Chromosome Compositions in *Trillium recurvatum*, Meeman-Shelby Forest, Shelby Co., Tennessee

Chromosome compositions of a natural population in *T. recurvatum* are shown by numbers of chromosome-banding patterns in A–E chromosomes. S marks indicate supernumerary chromosomes.

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Sample No.	Chromosome Composition						
1	A- 1· 1	B- 1· 1	C- 2· 2	D- 1· 1	E- 1· 1	S1	
2	A- 1· 1	B- 1· 2	C- 2· 2	D- 1· 2	E- 2· 2	S1	
3	A- 1· 1	B- 1· 2	C- 2· 3	D- 2· 2	E- 1· 1	S1	
4	A- 1· 1	B- 1· 3	C- 3· 4	D- 2· 2	E- 1· 5	S2	
5	A- 1· 1	B- 1· 7	C- 2· 2	D- 1· 2	E- 5· 6	S2,	S2
6	A- 1· 2	B- 1· 1	C- 1· 2	D- 1· 1	E- 1· 1	S1	
7	A- 1· 2	B- 1· 1	C- 2· 2	D- 1· 1	E- 1· 2	S1	
8	A- 1· 2	B- 1· 1	C- 2· 2	D- 1· 2	E- 1· 3	S2	
9	A- 1· 2	B- 1· 1	C- 2· 2	D- 1· 2	E- 4· 4	S2,	S2
10	A- 1· 2	B- 1· 1	C- 2· 2	D- 2· 2	E- 1· 1	S1	
11	A- 1· 2	B- 1· 2	C- 2· 2	D- 1· 1	E- 4· 4	S1	
12	A- 1· 2	B- 1· 3	C- 2· 2	D- 1· 1	E- 2· 2	S2	
13	A- 1· 2	B- 1· 6	C- 1· 2	D- 1· 2	E- 2· 2	S1,	S2
14	A- 1· 2	B- 2· 2	C- 2· 2	D- 1· 2	E- 2· 3	S1	
15	A- 1· 2	B- 2· 3	C- 2· 2	D- 1· 2	E- 1· 3	S1,	S2
16	A- 1· 2	B- 2· 4	C- 2· 3	D- 2· 2	E- 4· 5	S2	
17	A- 1· 2	B- 4· 4	C- 2· 2	D- 1· 2	E- 2· 7	S2	
18	A- 1· 2	B- 4· 5	C- 2· 2	D- 1· 1	E- 2· 2	S1,	S1
Total						18	

Kinds of chromosome-banding were two kinds in A chromosome, six in B chromosome, five in C chromosome, two in D chromosome and seven in E chromosome, with two kinds in supernumerary chromosomes. The B and E chromosomes are variable, while the A and E are stable (*T. kamtschaticum* is the most variable in the A chromosome and the most stable in the E chromosome. FUKUDA and KOZUKA, 1958, FUKUDA, 1970).

Characteristics of chromosome-banding in *T. recurvatum* is a large part of heterochromatin in the C chromosome, while there is a difference in the lengths of right and left arms in the E chromosome, although the other *Trillium* species have metacentric segments, there are sub-metacentric segments in *T. recurvatum* (Fig. 4). Its supernumerary chromosomes are both telocentric and acrocentric segments.

(2) Chromosome Compositions in A Population

Table 1 shows the chromosome composition of *T. recurvatum* in the Meeman-Shelby Forest, Shelby Co., Tennessee. The chromosome composition was comparatively homogeneous. The inbreeding coefficient,

Table 2. Inbreeding coefficient

Kinds of *Trillium* spaces		f
Pedicellate-flowered		
	Trillium kamtschaticum	0.0410
	Trillium erectum	0.2295
	Trillium grandiflorum	0.3866
Sessile-flowered		
	Trillium recurvatum	0.4237

$$f=\frac{\text{observed homozygotes }(\%)-\sum iPi^2}{1-\sum iPi^2}$$

where

Pi : population frequency of chromosome pattern Ai.

Zygotic proportions:

2 PiPj(1−f) in AiAj($i \neq j$),

f Pi+(1−f) Pi^2 in AiAi.

Table 3. Reproductive system of *Trillium* plants

Flowering Period	
Pedicellate-flowered *T. grandiflorum*	7 days
Sessile-flowered *T. recurvatum*	14 days
Pollen Fertility	
Pedicellate-flowered *T. kamtschaticum*	97.4%
Sessile-flowered *T. recurvatum*	85.2%
Ovule Formation	
Pedicellate-flowered *T. kamtschaticum*	288.08±20.62
Sessile-flowered *T. recurvatum*	22.83±2.45
Seed Formation	
Redicellate-flowered *T. kamtschaticum*	106.16±6.83
Sessile-flowered *T. recurvatum*	11.15±1.59
Seed/Ovule ratio	
Pedicellate-flowered *T. kamtschaticum*	0.37
Sessile-flowered *T. recurvatum*	0.49

f, was calculated on the basis of the chromosome-banding pattern (Table 2). When compared with other pedicellate-flowered species, *T. erectum*, *T. grandiflorum*, *T. ovatum*, this sessile-flowered *T. recurvatum* was found to have the highest value. That is, the plants of *T. recurvatum* are inbreeding.

Reproductive System in T. *recurvatum*

Table 3 indicates the reproductive systems of *Trillum* plants judging from the field observations. The *T. recurvatum* has a 14-day flowering period, although *T. gradiflorum* and *T. kamtschaticum* flower for only 7 days. The pollen fertility is 97.4% in *T. kamtschaticum*, while it is only 85.2% in *T. recurvatum*. The greatest difference was in ovule formation: it was 288 in a pedicellate-flowered *T. kamtschaticum*, while

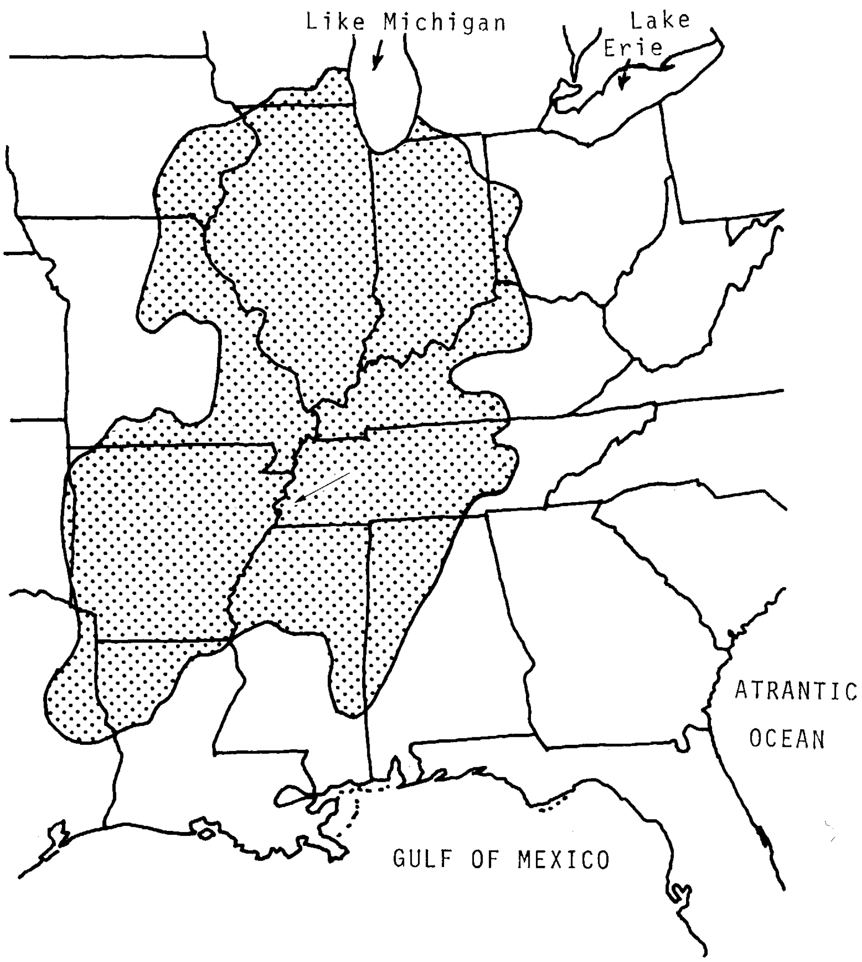

Fig. 3. Distribution map of *T. recurvatum* in eastern United States of America (FREEMAN, 1975). The arrow indicates the locality of the Meeman-Shelby Forest, Shelby Co., Tennessee from which materials were collected.

the sessile-flowered *T. recurvatum* was only 22.8. In terms of seed formation, *T. kamtschaticum* had 106 seeds in a fruit, but *T. recurvatum* had only 11 seeds.

In the flowering period of two weeks, the present author frequently visited the habitat of *T. recurvatum* and stayed all day to check for visiting insects. However, no such insects could be collected. As a result it was determined that no insects visited the flowers of *T. recurvatum* (FUKUDA, 1961). Meanwhile, in the flowering period the stamens easily touch the stigma (Fig. 2). The petals cover the stigma and stamens tightly.

It seems that such floral structures emphasize the inbreeding system and establish homogeneous chromosome composition in the population.

Moreover, it is very interesting that all plants of the population have one or two supernumerary chromosomes. They also might be the result of inbreeding which causes decreases in the pollen fertility and the seed production.

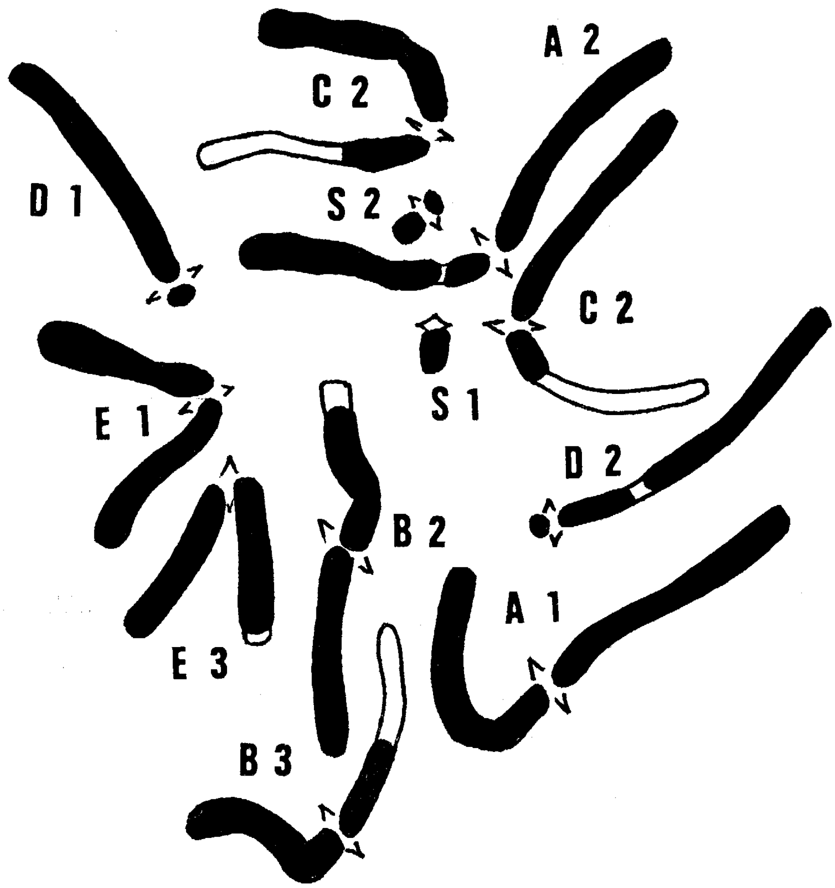

Fig. 4. Chromosome Types of *Trillium recurvatum* Beck. A drawing showing chromosome composition by chromosome-banding pattern numbers based on distinctions of the locations of heteromatin in a somatic metaphase.

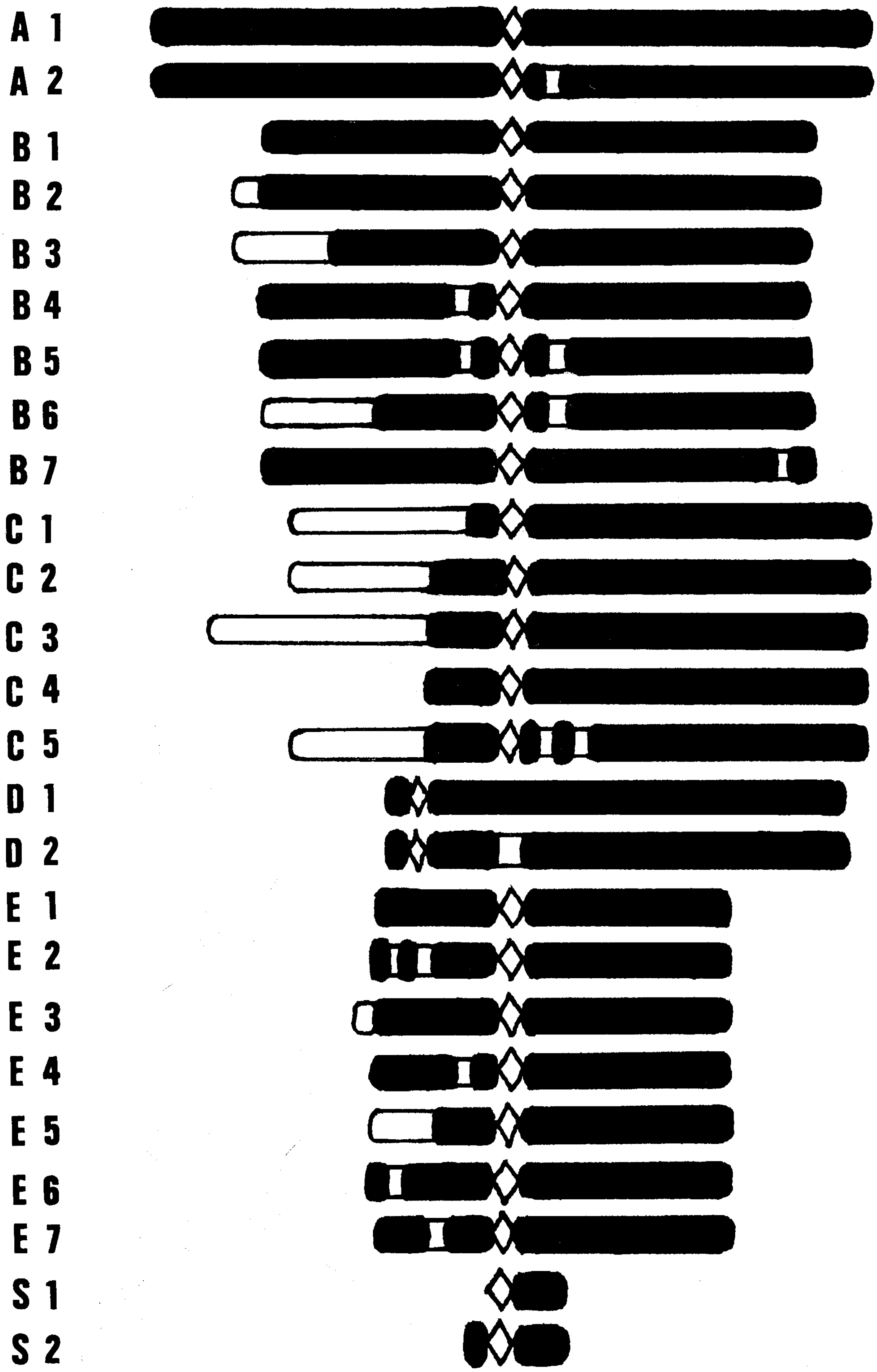

Fig. 5. Schematic drawings of chromosome-banding patterns in *T. recurvatum*.

Acknowledgements

This investigation was carried out during my teaching at Rhodes College, Memphis, Tennessee, as a distinguished visiting professor in the academic year of 1986–1987. I would like to express my gratitude to Associate Dean D. Robert R. Llewellyn and all members of the Department of Biology, Rhodes College. I also wish to thank President Paul T. Lauby of the United Board of Christian Higher Education in Asia in New York, for my financial support of the exchange-professor program.

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